Efficacy of anti-adhesion gel of carboxymethylcellulose with polyethylene oxide on peripheral nerve: Experimental results on a mouse model

EFFICACY OF ANTI-ADERENTIAL GEL OF CARBOXYMETHYLCELLULOSE WITH POLYETHILENE OXIDE ON PERIPHERAL NERVOUS SYSTEM: EXPERIMENTAL RESULTS ON A MICE MODEL

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Key words

CMC – PEO, perineural adherence, peripheral nervous system, recurrent compressive syndrome, antiadherential device

Running Title

Efficacy of a new CMC – PEO compound in the prevention of perineural scar formation.

Abstract

Object: Scar tissue formation around a nerve is the second most frequent reason of recurrence after nerve decompression in compressive neuropathies. This event is responsible of pain and loos of function of affected arm and, if no treated, irreversible injuries can arise. The surgeon has to perform neurolysis and to restore a correct gliding surface between treated nerve and surrounding tissues. In case of severe nerve compression recurrence local or free tissue transfer are required. In slight or mild case biocompatible anti adherential devices are required. This products present different biochemical composition, acting and shape. In this study we tested on a sciatic nerve mice model a gel composed by carbossy-methyl-cellulose (CMC) and polyethylene oxide (PEO) in order to describe its safety and efficacy.

Methods: Twenty-six adult mices underwent surgical procedure in which we burned the muscular bed of sciatic nerve bilaterally and in one of the nerves we applied anti adherential gel. After three weeks we measured the maximum force required to detach the nerve from the muscle through an apposite instrument and by histological evaluation of scar tissue with specific stain for collagen fibers.

Results: According to the results obtained by histological and biomechanical analysis the CMC-PEO gel is able to reduce perineural scarring. The group of burned muscle bed shows adhesion force of 46g, CMC-PEO gel group of 37g, control group 31g. There is a statistically significant difference between the gel group and burned group. Even histological analysis shows reduction of the scar tissue after application of gel.

Conclusions: Our preclinical animal model study shows that CMC-PEO gel can reduce perineural scar formation. In histological section the scar tissue arise also in gel group, but a gliding surface is identifiable between scar tissue and nerve and no direct connection between nerve and pathologic collagen fibbers are present. We didn't reported adverse effect or complication after surgery and this show completely biocompatibility and safety of tested product.

Introduction

Scar tissue formation between nerve and surrounding muscle is one of the most undesired occurrence in nerve surgery. Perineural scar tissue is responsible of recurrent compressive syndrome [1] both in peripheral nervous system (PNS) and in central nervous system (CNS) (e.g. nerve roots) [2, 3]. In PNS the perineural fibrosis is the second most frequent cause of recurrent carpal tunnel syndrome [4, 5]. These patients have to undergo to re operation due to invaliding symptoms that affect daily activity. The most frequent pathologies connected to this condition are traction neuropathy [6] and type II Complex Regional Pain Syndrome [7]. In order to prevent complete loos of function is mandatory to perform external neurolysis and then to bring gliding barrier on affected nerve. Vascularized tissue act well as gliding barrier, but harvesting local or free flaps is difficult and complications are described [8]. An alternative is cover treated nerve with biocompatible devices. These products have been developed and proposed in the last decades on animal models and then in surgical practice.

Initially, anti - adherential devices have been developed from films used in abdominal surgery in order to prevent adherential syndromes [9]. For peripheral nerve reparation, the classical form was not considered appropriate, thus a hydrogel form was developed. The biochemical composition has been modified too: collagen and dextran were introduced first [10], then Hyaluronic Acid (HA) both in experimental models and in clinical practice [11-14] as Hyaloglide (Fidia Advanced Biopolymers, Abano Terme, Italy). In late 2000, manifold studies employed carboxymethylcellulose (CMC) also associated with Phosphatidylethanolamine (PE) or Polyethilene oxide (PEO) [15-17] in order to improve anti adherential capacity.

This kind of anti adherential device was previously and successfully used in CNS as OXIPLEX®/SP Adhesion Barrier Gel (FzioMed, Inc. San Luis Obispo, CA, USA) or MEDISHIELDTM Adhesion Barrier Gel (Medtronic International Trading SARL, Tolochenaz, Switzerland) proving a reduction of epidural fibrosis in lumbar surgery [18] and minor pain and radiculopathy after laminectomy, laminotomy or discectomy [19]

The CMC-PEO formulation for PNS was not tested on preclinical in vivo models in order to describe its anti-adhesion potential and efficacy; hence we decided to build a model capable of measuring the effective utility of this device.

In this study we tested the efficacy of Dynavisc® (FzioMed Inc, San Luis Obispo), a CMC-PEO gel, in reduction of perineural scar tissue formation in a mice model by evaluating the peak pull out force [12] and histological aspect of the muscle-nerve interface before and after

gel application on an injured sciatic nerve model following methods described previously [20].

Methods

All procedures were performed in accordance with the Local Ethical Committee and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

In order to perform our analysis we employed a previous versatile, widespread and cost effective animal model recently described [20].

Twenty six Crl:CD1 (ICR) adults mices (5 weeks old, average weight 28g, Charles River Laboratories, Calco, Lecco, Italy) were anesthetized using a combination of 100 mg/kg of Ketamine and 15 mg/kg of Xylazine applied intraperitoneally. Under microscopic magnification, we exposed both sciatic nerves by gluteal splitting incision to view clearly the sciatic nerve from the gluteal vein to triforcation as presented in Fig. 1a.

Accordingly, we randomly divided all sciatic nerves into three experimental groups: burning group (1), burning + anti adherential gel CMC-PEO group (2), control group (3).

Burning group: after retraction of the nerve we burned the muscle surface with diatermocoagulator for about 0.8cm along the nerve bed (Fig 1b), as previously described [11, 20, 21]

Burning + anti adherential gel group: after muscle burning on muscular bed we applied a small quantity of gel (1ml) in order to completely cover and surround the nerve as illustrated in Fig 1c.

In control group we just exposed sciatic nerve and immediately we closed skin with 3-0 prolene sutures.

Animals were stabulated with standard light conditions with unlimited access to food and water. After 3 weeks all animals were sacrificed by cervical dislocation. In each group, biomechanical evaluation was performed. Three nerves for each group were not tested biomechanically and processed for histological evaluation.

Biomechanical evaluation was performed to measure the peak pull out force of the nerve from the muscular bed by method and tools described in a previous work [20]. Basically the tool consists in using a force applied constantly to the nerve until the traction breaks the adherences between the nerve and the surrounding tissue.

For histological analysis, the posterior space of the tight with nerve and scar tissue inside the muscles was harvested en bloc. The proximal end was marked with 9-0 Nylon. After paraffin inclusion [22] transversal sections (11μm thickness) were obtained and they were stained with Sirius Red following previously described protocol [23].

Statistical analysis of results was subjected to Student T-test. The significancy was established when $p<0.05$.

Results

Biomechanical analysis' results are resumed in Fig. 3 as the mean weight necessary to tear the nerve away from muscle. Statistical analysis (t-test) showed that burning muscular bed creates valid scar tissue (Burning vs Control $p < 0.001$). Moreover CMC-PEO gel application strongly reduces scar tissue (Burning + anti adherential gel vs burning $p = 0.003$).

Histological analysis showed different patterns of scar tissue formation. The sciatic nerve is stained in red such as the scar tissue surrounding epineurium. In the control group there is not scar tissue (Fig. 4a). In burned section, (Fig. 4b) perineural scar is strictly connected to muscle and penetrates epimysium with spicules that reach and surround muscle cells. In this way the nerve appears undetectable from the surrounding tissues. In burning + anti adherential gel group (Fig. 4c) is described a thinner scar layer compared to burning group that allows to the nerve to glide easily on muscular bed.

Discussion

In this paper we described the anti-adherential potential of CMC-PEO gel (FzioMed Inc, San Luis Obispo) specifically studied for peripheral nerve. The necessity to give gliding surface to the nerves is well known due to his excursion during limb movement as demonstrated by ex vivo studies[24, 25]. Scar tissue limits this physiological property leading to chronic ischemia of the nerve and intraneural scar formation that is responsible for traction neuropathy [6].

Many authors proposed different ways to obtain nerve protection and gliding using local soft tissues: vein wrapping [26], local adipose or muscular flap [27-29] or free omental flap [30]. These kinds of procedures have to be performed by experienced surgeon because they present complications and needed further nerve manipulation that leads to more scar stimulation (epineurium stretching, bleeding etc). Due to these, a recent paper [8] proposed local or free tissue transfer just in case of severe scar compression in plurioperated patients. In case of mild fibrous compression the authors proposed external neurolysis and anti adherential devices application that is easier and quicker to apply than tissue coverage.

Since late ’70s manifold methods and devices have been tested in order to prevent scar tissue formation after surgical procedures on central and peripheral nerve surgery. First studies were focused on scar tissue prevention on spinal root [2, 3] but in a few years also experimental protocol started on peripheral nerve [31] because perineural scar formation is one of the most frequent causes of peripheral nerve surgery failure [1] and is responsible of recurrent compression with new symptoms that requires adjunctive surgical procedures to restore nerve function.

Since 2000 the studies on peripheral nerve increased, with the development of new chemical barriers with different composition in comparison to previous devices. The first biocompatible device used in experimental models was ADCON-T/N® composed by Collagen and Dextran [10]. Numerous recent studies employed Hyaluronic Acid in different concentration and compositions [11-13] and they showed higher efficacy of this products. Those findings have been also confirmed by clinical studies on peripheral nerves [14]. However, cases of cerebrospinal fluid leakage after the use of carbohydrate polymer gel in spinal surgery have been reported. Bio-absorbable materials with reliable anti-adhesive effects that do not disturb healing of the surgical wound were clearly required, so next step for perineural scar prevention concerned the employment of Carboxymethylcellulose with Phosphatidylethanolamine a nonionic water-soluble polymer,(CMC-PEO) [15-17] that improve superficial gliding [32, 33].

CMC was initially employed in abdominal surgery and presented good results on peritoneal adhesions [34]. In peripheral nerve surgery this molecule was initially used as film [35] with good results as well on scar prevention on rabbit sciatic nerve. About CMC there are a lot of evidences of efficacy alone and composed with HA on animal models [11].

Yamamoto [15] associated to CMC a phospholipid surfactant-like substance called PE that in previous study presented gliding properties [36]. PEO, was added to CMC preparations since 2005 due to his biochemical properties. It seems that PEO inhibits the depositions of proteins on tissue surface [37, 38].

The CMC-PEO has been developed from the previous used Oxiplex Bioabsorbable Gel® (FzioMed Inc, San Luis Obispo) already used in central nervous system surgery. Sodium carboxymethylcellulose is a high molecular weight polysaccharide polymer that is water soluble, biocompatible, heat stable, and available in various molecular weights and viscosities [39]. PEO is a nonionic, water-soluble polymer widely used for stabilizing colloids and for formulating pharmaceuticals products. Fibrin and fibrin gel matrix do not interact well with PEO, limiting interaction between apposing surfaces; in particular PEO inhibits the deposition of proteins on tissue surface [37, 38]

In tested device CMC and PEO are combined with calcium chloride, forming an intramolecular CMC-carboxylate–calcium-chloride ion complex. This rheological structure alters the mobility of CMC and provides the interaction between the CMC and PEO, which ultimately determines the rheology, tissue adherence, and residence time. We didn't observe any reaction during and after the surgery, demonstrating the biocompatibility and safety of the product.

Our findings suggest that the CMC-PEO is a solid and valid tool to use to improve the gliding of the nerves after a surgical insult. Our model clearly shows a reduction of the scar tissue formation between the different layers form the nerve and the muscle around. This was texted both mechanically and histologically. The gliding effect of CMC-PEO device is emphasized by the histological sections in which scar tissue is present as a thin layer separated from the epineurium. Our findings seems to clarify the previous studies showed that CMC works just as mechanical barrier [18] and does not presents biochemical action on scar pathogenesis.

However, there are some limits to our study. With this animal model doesn't allow to understand if the improvement of the gliding means a better clinical outcome. We didn't investigate the symptomatology directly, but we observe a reduction of what the literature considered the main reason of the traction neuropathy and type II Complex Regional Pain

Syndrome. Our findings have to be confirmed with more randomized trials focused on the symptomatology. In fact, it's not possible to perform functional analysis in nerve compression model because the motor impairment is a late occurrence.

Other open claim is about what is the real mechanism of action of the CMC-PEO. Some authors think that anti-adherential properties are connected to barrier effect and it doesn't act biochemically [18]. In others studies, CMC and other similar biomaterials avidly attract and are engulfed by macrophages [40]. CMC is thought to suppress this action locally with a direct interaction of the Macrophages activate. Our study doesn't clarify that, but a future option could be focused on the reason of the inhibition the macrophages' activity.

Conclusions

In this study we applied anti-adherential gel on a burned perineural muscle bed that create a wide scar tissue. On this substrate we applied CMC-PEO gel and performed both biomechanical and histological analysis. Both evaluation methods showed clearly and statistically significant the reduction of scar tissue after gel application compared to burning group. Additional studies concerning the bimolecular actions of CMC-PEO and functional are needed because there are no clear evidences on their action mechanism. More over, it's not possible to perform functional analysis in nerve compression model because the motor impairment is a late occurrence. Although numerous studies exist in literature to test different anti-adhesion devices, nowadays does not exist a study that compares these products in order to identify which one is the most effective in peripheral nerve scar prevention. In conclusion, our study proves the efficacy, in animal models, of this new CMC-PEO gel in scar tissue formation prevention and discloses the absolute security and biocompatibility of this product.

Aknowledgements

None

Disclosure

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Figure Legends

Fig 1: a) gluteal splitting incision to expose sciatic nerve, b) burning muscular bed surrounding sciatic nerve, c) 1ml of CMC-PEO gel application after burning muscular bed.

Fig 2: a) schematic view of biomechanical evaluation tool. We measured the peak pull out force needed to de-touch the nerve from the muscular bed. The peak pull out force corresponds to scar duress. b) microscopical view of proximal sciatic nerv end connected to extraction tool.

Fig 3: Peak Pull Out Force for 3 groups. After CMC-PEO gel application we registered a significant reduction on scar tissue duress (37,86g in CMC-PEO gel group vs 46,57g in burning group).

Fig 4: Histological view of transvers section of en bloc withdrawal (Sirius Red Stain, 10x). In a normal aspect of sciatic nerve (S) and his surrounding muscles (M) without pathological scar tissue. After burning of muscle bed (b), scar tissue in identifiable (*), collagen fibers spiculae (□) strictly connect nerve to surrounding tissue. After CMC-PEO gel application on burned muscular bed (c) scar tissue in also appreciable (*), but appears thinner than scar tissue in previous section and is separated from epineurium (E). A cleavage plane (§) is identifiable between scar tissue and epineurium and it allows free gliding of the nerve during limb movement and confirms biomechanical evaluation's results. More over no spiculae connect scar tissue strictly to muscle bed.